Histamine regulates a variety of physiological processes including inflammation, gastric acid secretion, and neurotransmission. The cellular response to histamine is subject to dynamic control, and exaggerated histamine reactivity in response to cysteinyl leukotrienes and other stimuli is important in a variety of different pathological conditions. The molecular mechanisms controlling histamine responsiveness are still unresolved. In investigating histamine responses in embryonic stem (ES5) and F9 embryonic carcinoma cells, we encountered a novel mechanism controlling the cellular reaction to histamine. Unstimulated cells displayed neither high affinity ([3H]pyrilamine binding nor histamine-induced increases in cytosolic Ca^{2+} levels. Pretreatment of these cells, however, with leukotriene D_{4}, leukotriene E_{4}, serotonin, or fetal calf serum induced an immediate and transient ability of these cells to respond to histamine with an increase in cytosolic Ca^{2+} levels. This effect could be inhibited by pertussis toxin and was mimicked by GTP analogues. Importantly, the latter compounds also provoked immediate high affinity ([3H]pyrilamine binding. We conclude that in these cells histamine responsiveness is directly controlled by pertussis toxin-sensitive G protein-coupled receptors, whose activation enables the H_{3} receptor to bind its ligand. These findings define a novel mechanism for regulating histamine H_{3} receptor activity and provide for the first time molecular insight into the mechanism by which cysteinyl leukotrienes and other external stimuli can increase histamine responsiveness.

Histamine, a biogenic amine formed by decarboxylation of the amino acid L-histidine (1), is found in large quantities in most tissues, mainly in the granules of mast cells, although numerous other cell types are capable of histamine synthesis as well (2). Histamine controls a multitude of physiological functions by activating specific receptors on target cells. Three types of receptors for histamine have been described, denominated as the H_{1}, H_{2}, and H_{3} receptor and are distinguished on the basis of their sensitivity to specific agonists and antagonists (3). In general, the H_{2} receptor is implicated in autoinhibition of histamine synthesis and release and the H_{3} receptor in gastric acid secretion, whereas the H_{1} receptor is involved in inflammatory responses, mediating for instance blood vessel and bronchial constriction, vascular permeabilization, and synthesis of other inflammatory agents (4). Histamine receptors are subject to dynamic regulation, receptor activity being increased or diminished in response to various conditions (5–7), and exaggerated histamine reactivity is associated with a variety of pathological disorders.

Cysteinyl leukotrienes have been implicated in the stimulation of histamine reactivity. Inhalation challenge with these inflammatory eicosanoids increases histamine responsiveness of the airways (8–12), and cysteinyl leukotriene-induced histamine hypersensitivity is presumed to be important in asthmatic disease (13). Also, other signaling molecules stimulate histamine responsiveness. Especially serotonin, platelet-activating factor, and thromboxanes are known to enhance histamine reactivity (14, 15). The molecular mechanisms, however, by which such stimuli can provoke increased histamine responsiveness have remained obscure.

In the present study we describe a molecular mechanism by which external stimuli can enhance histamine reactivity by directly controlling the affinity of the histamine H_{3} receptor for its ligand. We have reported earlier that the P19 embryo carcinoma (EC) cell, a pluripotent cell type resembling the inner cell mass of the embryo, expresses functional histamine H_{3} receptors (16), although its function with respect to embryogenesis is not clear. To obtain more insight into the function of histamine receptor expression in uncommitted cells, we decided to investigate the presence of cellular responses to histamine in other pluripotent cells. We observed that F9 EC cells and embryonic stem (ES5) cells displayed neither high affinity ([3H]pyrilamine binding nor histamine-induced increases in cytosolic Ca^{2+} levels. A pretreatment of these cells with cysteinyl leukotrienes, serotonin, or FCS, however, induced an immediate and transient ability of these cells to react to histamine. This effect was inhibited by pertussis toxin and was mimicked by GTP analogues. Importantly, induction of histamine responses coincided with the appearance of high affinity ([3H]pyrilamine binding sites on these cells. Apparently pertussis toxin-activating agents can regulate histamine responses by inducing high-affinity binding sites for histamine. These findings define for the first time a molecular mechanism by which cysteinyl leukotrienes and other external stimuli can increase histamine responsiveness and identify a novel mechanism for the regulation of G protein-coupled receptors.
EXPERIMENTAL PROCEDURES

Chemicals—Histamine dihydrochloride, pyrilamine (maleate salt), leukotrienes, serotinin, and valinomycin were obtained from Sigma. Fura-2 acetoxymethyl ester was from Molecular Probes (Eugene, OR). Streptolysin O was from Wellcome Diagnostics (Dartford, UK). GTP-S was from Boehringer Mannheim, okadaic acid was from Life Technologies, Inc., and [pyrrolidinyl-5]-Histamine ([\(^{3}H\)]histamine) was from Amersham (Buckinghamshire, UK). The enantiomers of cicletanine were kind gifts from the Henri Beaufour Institute-IPSEN Laboratories, France. SH-FCS was prepared at our laboratory by DTT treatment of fetal calf serum. DTT hydrolyzes the protein S-S bridges and thereby inactivates most of the polypeptide growth factors in FCS. The thus-treated serum is dialyzed to remove traces of DTT.

Cell Culture—F9 EC and P19 EC cells were cultured at 7.5% CO\(_2\) and 37 °C in bicarbonate-buffered DF-medium supplemented with 7.5% FCS. ES5 and D3 ES cells were maintained in conditioned minimal Eagle’s medium containing 4 nM \[^{3}H\]pyrilamine and different concentrations and intact cells was performed as described earlier (16). For whole-cell patch clamp analysis, cells were maintained in serum-free medium for 1 h and subsequently loaded with 10 \(\mu\)M Fura-2 acetoxymethyl ester at 33 °C. For experiments with NaF (20 mM) and AlCl\(_3\) (75 mM), cells were washed three times with phosphate-buffered saline and cell protein was precipitated with 0.2 M NaOH. The bound radioactivity was determined by liquid scintillation counting. In each experiment, each condition was tested in triplicate. For experiments with NaF (20 mM) and AlCl\(_3\) (175 \(\mu\)M), the same buffer was used as described for Ca\(^{2+}\) determinations and applied for 20 min at room temperature, after which the cells were placed on ice and the \[^{3}H\]pyrilamine binding assay was performed. For experiments with GTP-S, cells were first permeabilized for 5–10 min at 37 °C with 0.5 IU/ml streptolysin O in 100 mM KCl, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 1.3 mM CaCl\(_2\), 2 mM EGTA, 0.1 mM MgCl\(_2\), 1 mM ATP, 10 mM Hepes, pH 7.2 (20) and washed once in the absence of streptolysin O. GTP-S was added to the permeabilized cells and incubated shortly (1–2 min) at room temperature to allow GTP-S to enter and sensitize the cells. Thereafter cells were placed on ice and labeled as described above. For permeabilized cells, the incubation buffer contained 150 mM KCl, 5 mM NaCl, 5.6 mM glucose, 1 mg/ml bovine serum albumin, 1 mM ATP, 1.3 mM CaCl\(_2\), 0.1 mM MgCl\(_2\), 2 mM EGTA, 10 mM Hepes, pH 7.2.

In general, Scatchard plots made using intact cells show considerable nonspecific low affinity binding of \[^{3}H\]pyrilamine (16), more so in permeabilized cells when compared with nonpermeabilized cells. Therefore, Scatchard plots were fitted according to a one- or two-site model, using the formula, binding/free = \(\frac{[\text{B}_{\text{max}1} - \text{bound}]}{[\text{K}_{\text{d}1}] + \frac{[\text{B}_{\text{max}2} - \text{bound}]}{[\text{K}_{\text{d}2}]} + 0.5 \cdot \frac{[\text{B}_{\text{max}1} - \text{bound}]}{[\text{K}_{\text{d}1}] + \frac{[\text{B}_{\text{max}2} - \text{bound}]}{[\text{K}_{\text{d}2}]} + 4 \cdot \frac{[\text{B}_{\text{max}1} - \text{bound}]}{[\text{K}_{\text{d}1}] + \frac{[\text{B}_{\text{max}2} - \text{bound}]}{[\text{K}_{\text{d}2}]}})\right)^2\), in which \(\text{B}_{\text{max}1}\), \(\text{B}_{\text{max}2}\), \(\text{K}_{\text{d}1}\), and \(\text{K}_{\text{d}2}\) are the respective maximal binding capacities and dissociation constants of the different affinities. The observed points of the Scatchard plot of unstimulated cells were satisfactorily fit with a one-site (low affinity) model, whereas two affinity binding sites could be distinguished in the sensitized cells. To determine best fit, we calculated the \(x^2\) distribution of the estimated curve relative to the observed values. We accepted the fit if the \(x^2\) did not exceed the probability value of 5%.

RESULTS

Induction of Histamine Signal Transduction in F9 EC and D3 ES Cells—Different EC and ES cell lines showed marked differences in their reaction toward histamine (1 \(\mu\)M). P19 EC cells and ES5 cells responded to histamine with a marked increase in cytosolic Ca\(^{2+}\) levels and transmembrane currents, as assayed with whole-cell patch clamp electrophysiology and fluorimetric Ca\(^{2+}\) determinations (Figs. 1 and 2). Such responses, however, were absent in the F9 EC and D3 ES cells (Figs. 1 and 2, Tables I and II). Even digital image analysis (which allows detection of small responses in single cells) of Fura-2-loaded F9 EC and ES5 cells did not reveal any response to histamine in these cells (Fig. 3). Importantly, we noted that stimulation of F9 EC and D3 ES cells with 5% (DTT-treated) fetal calf serum (SH-FCS), 1 \(\mu\)M leukotriene D\(_4\), 1 \(\mu\)M leukotriene E\(_4\), or 3 \(\mu\)M serotonin induced an ability in these cells to respond to histamine: after prestimulation with one of these compounds, both histamine-induced Ca\(^{2+}\) responses and transmembrane currents were easily detected (Figs. 1–3, Tables I and II). Control experiments consistently showed that F9 EC and D3 ES cells spontaneously reacted toward ATP (50 \(\mu\)M) and bradykinin (1 \(\mu\)M) but never did show uninduced histamine responsiveness (\(n = 29\)). Furthermore, ATP and bradykinin were not able to induce histamine responsiveness. We were confident, therefore, to have encountered a novel form of regulation of histamine responsiveness, as the cellular reaction to histamine in the F9 EC and D3 ES lines requires sensitization by specific stimuli.

Histamine Responsiveness Induced by SH-FCS Is Transient

**FIG. 1.** The effect of histamine (HA) on the intracellular Ca\(^{2+}\) concentration in P19 EC and F9 EC cells. Representative traces of the intracellular Ca\(^{2+}\) concentration of P19 Fura-2-loaded P19 EC (A) and F9 EC (B and C) are shown. The additions of the stimuli are indicated.

Histamine responsiveness, as the cellular reaction to histamine in the F9 EC and D3 ES lines requires sensitization by specific stimuli.
To further characterize the induction of histamine responsiveness, we performed fluorimetric Ca\(^{2+}\) determinations in the F9 EC cell line using SH-FCS as a pre-stimulus. The induction of histamine responsiveness by SH-FCS is fast, as coapplication of SH-FCS and histamine had a supra-additive effect. The SH-FCS-induced responsiveness, however, is of a highly transient nature. Already, 10 min after application of serum, the sensitivity of these cells to histamine was lost (Fig. 4). Pharmacological studies carried out after induction of histamine responsiveness with 5% SH-FCS showed that the reaction to histamine in these cells was inhibited by the H1 receptor antagonists pyrilamine (1 \(\mu M\)) and (+)-cicletanine (15 \(\mu M\)) but not by the H1-unspecific enantiomer (-)-cicletanine (15 \(\mu M\)). Apparently, SH-FCS transiently enables H1 receptor signaling in D3 ES and F9 EC cells. Subsequent experiments were performed to obtain insight into the mechanisms implicated in the regulation of this transient histamine H1 receptor responsiveness.

**Role of the Ca\(^{2+}\) Response in the Induction of Histamine Responsiveness**—Although leukotrienes and serotonin activate only minor Ca\(^{2+}\) fluxes when compared with SH-FCS, histamine-induced Ca\(^{2+}\) responses were not different after induction with either leukotrienes, serotonin, or SH-FCS (Fig. 4B; Table II). Also, stimulation with FCS, which tends to yield bigger Ca\(^{2+}\) responses when compared with SH-FCS (not shown), did not produce different Ca\(^{2+}\) responses to histamine. These results suggest that the size of the Ca\(^{2+}\) response produced by the sensitizing stimulus is not indicative of the

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**TABLE I**

<table>
<thead>
<tr>
<th>Condition</th>
<th>F9 EC</th>
<th>ES5</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mM histamine</td>
<td>0/29</td>
<td>0/12</td>
</tr>
<tr>
<td>5% SH-FCS + 0.1 mM histamine</td>
<td>66/84</td>
<td>20/20</td>
</tr>
<tr>
<td>100 ng/ml pertussis toxin + 5% SH-FCS + 0.1 mM histamine</td>
<td>0/7</td>
<td>ND</td>
</tr>
<tr>
<td>20 mM NaF + 75 (\mu M) AIF(_3) + 0.1 mM Histamine</td>
<td>10/20</td>
<td>ND</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of responses</th>
<th>Histamine-induced current pA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 mM histamine</td>
<td>0/15</td>
<td>---</td>
</tr>
<tr>
<td>5% SH-FCS + 0.1 mM histamine</td>
<td>9/9</td>
<td>90 ± 50</td>
</tr>
<tr>
<td>1 (\mu M) LTD(_4) + 0.1 mM histamine</td>
<td>4/4</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>1 (\mu M) LTE(_4) + 0.1 mM histamine</td>
<td>6/7</td>
<td>80 ± 50</td>
</tr>
<tr>
<td>3 (\mu M) serotonin + 0.1 mM histamine</td>
<td>4/4</td>
<td>120 ± 80</td>
</tr>
<tr>
<td>100 ng/ml pertussis toxin + 5% SH-FCS + 0.1 mM histamine</td>
<td>0/5</td>
<td>---</td>
</tr>
<tr>
<td>100 ng/ml pertussis toxin + 1 (\mu M) LTE(_4) + 0.1 mM histamine</td>
<td>0/4</td>
<td>---</td>
</tr>
</tbody>
</table>
amount of subsequently induced histamine responsiveness. To
test this possibility, we initiated a series of experiments in
which the size of the Ca$_{2+}$ response to SH-FCS was compared
with the Ca$_{2+}$ response to histamine added 60 s later. As shown
in Fig. 4C, no relationship between the two Ca$_{2+}$ responses was
detected. We concluded that the induction of histamine respon-
siveness is independent of the size of the prior Ca$_{2+}$ response
provoked by the sensitizing agent.

Induction of Histamine Responsiveness in F9 EC Cells Re-
quires Pertussis Toxin-sensitive G Proteins—

To further inves-
tigate the signal transduction pathways regulating this tran-
sient histamine responsiveness, we observed that an increase
in intracellular Ca$_{2+}$, cAMP analogues or forskolin treatment,
cGMP analogues, inhibitors of phospholipase A$_2$, arachidonic
acid, or the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (acute or overnight treatment) neither induced nor pre-
vented the sensitization of histamine responses in F9 EC cells
(not shown). Because serum, leukotriene D$_4$, leukotriene E$_4$, and serum activate pertussis
toxin-sensitive G proteins, we investigated the effect of a 4-h
pretreatment with pertussis toxin (100 ng/ml). It appeared that
the serum-, leukotriene- and serotonin-provoked inductions of
histamine responsiveness were abolished by this procedure (as
determined either by fluorimetric Ca$_{2+}$ determinations or
patch clamp electrophysiology; Tables I and II). Therefore, the
sensitization of the histamine response appears to be depend-
ent on the activation of pertussis toxin-sensitive G protein-
coupled receptors. To test whether activation of G proteins is
sufficient for induction of histamine responsiveness, F9 EC
cells were injected with 10 mM GTP$_\gamma$S. This procedure was
indeed sufficient for inducing histamine responsiveness, as
assayed with whole-cell patch clamp (Fig. 5B), whereas GDP/βS
injection did not produce this effect. The GTP$_\gamma$S-induced his-
tamine responsiveness was, however, of a highly transient

![Fig. 4](image-url)
nature (Fig. 5C), maybe due to GTPγS-dependent activation of histamine signaling elements, making further stimulation of these signaling elements by the receptor impossible. In agreement, impalement of cells with GTPγS-containing pipettes provoked strong currents, indicating activation of such signaling elements by GTPγS. GTPγS-induced histamine responsiveness was eliminated by the H1 receptor antagonist pyrilamine (Fig. 6) but not by cimetidine (a H2 receptor antagonist), demonstrating that this histamine responsiveness is mediated by the H1 receptor. Treatment of cells with 20 mM NaF and 75 μM AlCl3 (which potently activates G proteins) led within 5 min after application to a slow but sustained increase in intracellular Ca2+ (Fig. 5A), probably due to activation of Ca2+-mobilizing G protein-dependent signaling elements. Importantly, such a treatment also induced histamine-dependent Ca2+ responses on top of the aforementioned sustained increase in intracellular Ca2+ levels within 20 min after application of AlF3 (Fig. 5A; Table I). We concluded that activation of pertussis toxin-sensitive G proteins is implicated in the induction of histamine responsiveness.

Induction of Histamine Responsiveness Coincides with the Appearance of High Affinity [3H]Pyrilamine Binding—To further explore the processes underlying the induction of histamine responsiveness, Scatchard analysis was performed. Surprisingly, in unstimulated F9 EC cells, no high affinity [3H]pyrilamine binding was observed (n = 7; Fig. 7A), in contrast to P19 EC cells (which react unconditionally to histamine), which exhibited high affinity binding of [3H]pyrilamine (kD ~ 7 nM; n = 2). In accordance, whole cell membrane preparations of P19 EC cells displayed high affinity binding of [3H]pyrilamine, but no such binding could be detected in F9 EC cells. These results suggest that the failure of F9 EC cells to react to histamine under uninduced conditions is due to the absence of high affinity histamine binding activity, and that induction of histamine responsiveness is caused by a rapid increase of high affinity histamine binding sites on the plasma membrane. Indeed, introduction of GTPγS into the cells rapidly induces [3H]pyrilamine binding with a Kd of 19 ± 4 nM and a Bmax of 0.15 ± 0.02 pmol/106 cells (± S.E.; n = 3; Fig. 7). Also, treatment of nonpermeabilized cells with 20 mM NaF and 75 μM AlCl3 induces high affinity [3H]pyrilamine binding with an apparent Kd of 24 ± 3 nM and a Bmax of 0.31 ± 0.12 pmol/106 cells (n = 4; Fig. 7B). As our observations with regard to the transient nature of the induced histamine responsiveness predicted a transient induction of [3H]pyrilamine by external sensitizing stimuli like leukotriene and AlF3, we also tested whether such transient [3H]pyrilamine binding to cells could be demonstrated. At 37 °C, these experiments yielded rather variable results, probably because only after 1 h of labeling, [3H]pyrilamine binding to cells reaches equilibrium, whereas induction of histamine responsiveness at this temperature by such stimuli is short-lived. When experiments were performed at lower temperatures, however, transient induction of [3H]pyrilamine binding became apparent (Fig. 8). Together our observations strongly suggest that a pre-stimulus-induced change in receptor conformation, resulting in a highly increased affinity for histamine, underlies the observed regulation of H1 receptor action in F9 EC cells. Therefore, these results define a hitherto undescribed mechanism controlling H1 receptor function.

DISCUSSION

In the present study we show that H1 receptor signaling in F9 EC cells and ES5 cells required prestimulation of the cells with either fetal calf serum, serotonin, leukotriene D4, or leukotriene E4, whereas bradykinin and ATP did not produce this effect. This sensitizing effect was inhibited by pertussis toxin, whereas it was mimicked by GTPγS and AlF3. Therefore, pertussis toxin-sensitive G proteins are probably mediating this regulation of H1 receptor action. The molecular basis for the induction H1 receptor responsiveness appears to be the appearance of high affinity ligand binding sites, as control cells did not display high affinity [3H]pyrilamine binding, but introduction of GTPγS or treatment with AlF3 immediately provoked such high affinity [3H]pyrilamine binding sites. These findings strongly suggest that histamine responses in these cells are controlled by agents that induce high affinity binding sites for histamine and define a molecular mechanism by which external stimuli can control histamine H1 receptor action.

The molecular details, however, by which external stimuli enable the H1 receptor to interact with its ligand, remain unclear. An explanation for the impaired H1 receptor function in unstimulated cells may be a physical impossibility for histamine to interact with its receptor. Generally, receptors may be continuously recycled between plasma membrane and endosomes. Although this process has not been reported for H1 receptors, it has been found to occur with several other G
protein-coupled receptors (e.g. Ref. 22), including the H_2 receptor (7), and some of the signaling elements involved have been identified (e.g. Refs. 23 and 24). It is conceivable that in cell types that require sensitization for histamine responsiveness, the balance between endosomal and plasma membrane localization is shifted to the endosomal state, and that sensitization releases this shift. Such a scheme would imply that in unstimulated F9 EC and ES5 cells the large majority of histamine receptors has an endosomal location. In other cells types, however, which show unconditional histamine responses, the balance between endosomal and plasma membrane-localized receptors should be shifted in favor of a plasma membrane location. In this context it is interesting to note that pertussis toxin-sensitive G proteins have been implicated in the stimulation of vesicle fusion (e.g. Refs. 25–27) and that rab3-mediated exocytosis from mast cells is pertussis toxin-sensitive (28). Such a mechanism, however, can not explain the absence of high affinity binding of [3H]pyrilamine in whole cell membrane preparations of uninduced F9 EC cells, prompting alternative explanations for induction of histamine responses in these cells.

Therefore, control of the histamine response by SH-FCS, leukotrienes, and serotonin may be mediated by the induction of a conformational change of the histamine H_1 receptor, resulting in increased ligand affinity. Our experiments using the phosphatase inhibitor okadaic acid in F9 EC cells implicate a serine/threonine phosphorylation event in the stimulus-dependent sensitization of the histamine response, opening the possibility that a phosphorylation of the receptor underlies this conformational change. In agreement, mutational analysis of the H_2 receptor has shown that relatively small changes in the primary sequence of the H_1 receptor can have profound influences on ligand affinity (7), and the primary sequence of the H_1 receptor contains a number of serine and threonine residues that may serve as potential phosphorylation sites for an affinity controlling mechanism because for stimulation of the H_1 receptor in these cells, a pre-stimulus as well as histamine are necessary. Interestingly, in pathological conditions like asthma and allergy, exaggerated histamine reactivity is associated with the formation of cysteinyl leukotrienes (13) and serotonin (15), but no molecular details are known. The findings described in this study for inducing histamine responsiveness define for the first time a molecular mechanism by which such control of hista-
mine reactivity may be exerted, but further studies are re-
quired to assess the importance of this mechanism in these
pathological conditions. These studies are currently under
progress.

Acknowledgments—The authors thank Gert Folkers and the other
members of our laboratories for stimulating discussions.

REFERENCES